Observations on the anatomy of the scaphopod mantle and the description of a new family, the Fustiariidae

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Abstract. The mantle of five genera of the order Dentaliida and ten genera of the order Gadilida are investigated using histological and ultrastructural methods. The anterior mantle edge is divided into three functional units: 1) a frontal epithelium; 2) a central fold; 3) an inner gland region. In the Dentaliida the frontal epithelium is differentiated as an outer gland region and the central fold carries an annular ciliary organ. *Rhabdus rectius* (Carpenter) (Dentaliida) has a pair of ciliated slits instead of the annular organ. Neither feature is found in the Gadilida, where the frontal epithelium forms sensory papillae. Different types of ciliary receptors in the anterior mantle edge of both orders are present. The inner gland region in the Dentaliida consists of epithelial gland cells only; in the Gadilida both epithelial and subepithelial gland cells are present. The epidermis of the pallial cavity is low cuboidal but features ciliary rings in the anal region.

The posterior pallial edge or pavillon of the gadilids has a powerful ciliary organ to produce water currents and ciliated ridges on the dorsal mantle process; both structures are lacking in the dentaliids. *Fustiaria* differs from other genera of the Dentaliida by lacking a ventral bolster of connective tissue at the posterior mantle margin. Abundance and distribution of subepithelial gland cells vary between genera. The validity of the family Rhabdidae is supported and a new family, the Fustiariidae, is proposed.

The anatomy of the Scaphopoda has received the least attention of all of the classes of the molluscan subphylum Conchifera. Early accounts, e.g. Deshayes, 1825; Lacaze-Duthiers, 1856-7; Fol, 1889; Plate, 1892; and Simroth, 1894, however, contained excellent studies of scaphopod morphology and histology that remain valid in many aspects. Although almost all investigations were made on a single genus, *Dentalium (=Antalis)* (order Dentaliida), the results have been generalized subsequently for all Scaphopoda.

Lacaze-Duthiers (1856-7), Plate (1892), and Boissevain (1904) described the histological structure of the mantle in *Antalis entalis* (L.). They noted an outer glandular region at the anterior mantle edge, including two to three types of gland cells, a ring of connective tissue supporting the mantle opening, and an inner glandular region consisting of mucus-producing cells. Plate (1892) found that the genus *Cadulus* lacked the outer gland region, and later Odhner (1931) confirmed. Boissevain (1904) reported a ring of ciliated cells between the gland regions that she considered to be a sensory organ. Gabe and Prenant (1950) included the anterior mantle edge of *A. entalis* in their histological studies of the scaphopod connective tissue.

In the midregion of the animal, ciliated ridges were found just anterior to the anal opening in *Antalis entalis* and *A. dentalis* (L.) by Lacaze-Duthiers (1856-7) and Fol (1889). Distaso (1906) described them in more detail and also claimed the presence of an osphradial sense organ. Leon (1895) and

Boissevain (1904) described the posterior mantle edge, the so-called pavillon, and reported a small ciliated area and scattered gland cells. Reynolds (1988) investigated sensory cells fo the pavillon of *Rhabdus rectius* (Carpenter) (Dentaliida).

This paper presents a comparative anatomy of the mantle in selected members of the orders Dentaliida and Gadilida (= Siphonodentaliida) to demonstrate the anatomical diversity in Scaphopoda and to provide new information for a better founded classification.

MATERIALS AND METHODS

The species studied are listed in Table 1. Mediterranean species were collected by means of SCUBA or by a triangle dredge from 8-20 m in Rovinj and Piran (Peninsula of Istria, Yugoslavia). In Norway, animals were gathered by a "Mini-Sanders" epibenthic sledge and box-core sampler from 120-590 m in the fjords near Blomsterdalen, Bergen. The animals from Puget Sound, Washington, U.S.A., were collected with a triangle dredge from 75-100 m near Waldrun Island.

HISTOLOGICAL PREPARATION

Living animals were narcotized with isotonic $MgCl_2$ solution (1:2 to 1:1 with sea-water) or MS 222 added to the sea-water (1:4000 - 1:2000). Tissues were fixed in 2-4% formalin or 4% glutaraldehyde buffered in sea-water. Helly's

Table 1. Systematic list of the species examined (USNM, United States National Museum of Natural History, Smithsonian Institution; NMNZ, National Museum of New Zealand; [1]Museum National d'Histoire Naturelle, Paris; [2]Gareth Davies, University of Edinburgh; [3]Zoologisk Museum, Kopenhagen; *prepared for TEM methods)

Order DENTALIIDA

Family	Source
Dentaliidae	
Dentalium laqueatum Verrill, 1885	USNM 765278
D. neohexagonum Sharp and Pilsbry, 1897	Monterey Bay
Graptacme calamus (Dall, 1889)	USNM 801250
Antalis dentalis (L., 1767)	Rovinj*
A. entalis (L., 1758)	MBI[2]
A. inaequicostatum (Dautzenberg, 1891)	Piran*
A. occidentalis (Stimpson, 1851)	Bergen, Norway*
A. vulgaris (Dacosta, 1778)	Rovinj
A. sp. BS 660	NMNZ BS660
A. sp. P 927 II	NMNZ P927 II
A. sp. 930	NMNZ P930
A. sp. Q 719	NMNZ Q719
Fissidentalium candidum (Jeffreys, 1877)	AT 186[2]
F. majorinum (Mabille and Rochebrune, 1889)	USNM 709081
F. megathyris (Dall, 1899)	Galathea St716[3]
F. zelandicum (Sowerby, 1860)	NMNZ BS496
Fustiaria rubescens (Deshayes, 1825)	Piran
F. sp.	NMNZ sand lagoon
Rhabdidae	
Rhabdus rectius (Carpenter, 1864)	San Juan Islands USNM 678797
Laevidentaliidae	
Laevidentalium callipaplum (Dall, 1889)	USNM 765451
Entalinidae	
Entalina quinquangularis (Forbes, 1843)	Thalassa Y 378[1] Bergen, Norway*
Bathoxiphus ensiculus (Jeffreys, 1877)	Incal WS 01[1]
B. sp. S 153	NMNZ S153
Heteroschismoides subterfissum (Jeffreys, 1877)	Nordatlante 85[1] ES 218[2]
Pulsellidae	
Pulsellum lofotense (M. Sars, 1865)	Bergen, Norway*
P. salishorum Marshall, 1980	San Juan Islands
P. sp. BS 940	NMNZ BS940
P. sp. P 937 I	NMNZ P937 I
Annulipulsellum euzkadii Scarabino, 1986	BIOGAS IV, DS63[1]
Striopulsellum sandersi Scarabino, in lit.	Nordatlante P19[1]
Siphonodentaliidae	
Siphonodentalium grandis (Verill, 1884)	Incal WS 02[1]
S. lobatum (Sowerby, 1860)	ESI64[2]
S. spectabilis Verill, 1885	Incal DS 09[1]
S. vitreum M. Sars, 1851	Greenl. Exp. 42[3]
Wemersoniellidae	
Wemersoniella turnerae Scarabino, 1986	Incal WS 07[1]
Gadilidae	
Gadila fraseri Nickles, 1979	Galathea St. 101[3]
G. metivieri Scarabino, in lit.	BIOGAS V, DS66[1]
G. sp.	NMNZ P939
Cadulus aberrans Whiteaves, 1887	Monterey Bay
C. arctatus Jeffreys in Locard, 1898	Thalassa Z 407[1]
C. cylindratus Jeffreys, 1877	ES 27[2]
C. delicatulus Suter, 1913	NMNZ BS544
C. jeffreysi (Monterosato, 1875)	BIOGAS V, DS66[1]
C. propinquus Sars, 1878	BIOGAS VI, DS82[1]
C. subfusiformis (M. Sars, 1865)	Bergen, Norway*
C. sp. 68	USNM 803468
C. sp. P927 I	NMNZ P927 I

fixative produced good fixation of cytoplasmatic components in small specimens and pieces of tissue. Bouin's fluid (aq.) penetrates tissues rapidly, decalcifies, but often damaged ciliation. This fluid was used also for decalcification after one of the other fixatives. Fixation times ranged from 2-24h. The specimens were washed in 70% ethanol + NH_4 (3 drops/l00ml) to remove the picric acid. After dehydration in ethanol, tissues were transferred to methyl benzoate as an antemedium, benzol, 40% paraffin and embedded in 58-60°C Paraplast. Sections of 5-7 μm were taken and stained with Azocarmine and Anilineblue-Orange G after Heidenhain (AZAN), Haematoxilin and Eosin after Mayer (HE) or Kernechtrot + Picroindigocarmine (KP). Recipes for fixation and staining were modified after Adam and Czihac (1964). Museum samples, usually kept in alcohol, were treated in the same way starting with decalcification or dehydration.

SEM PREPARATION

The same fixatives were used as for histological purposes. Tissues were dehydrated in grades of acetone, critical point dried with liquid CO_2 and sputter coated with a 200 Å gold-layer. The samples were examined in a Jeol JMS 09 scanning electron microscope.

TEM PREPARATION

Two methods were applied as follows: 1) 4 hr in 3% glutaraldehyde in phosphate buffer 0.1M pH 7.2-7.4 (after Sorensen) or cacodylate buffer 0.1M pH 7.2; postfix for 2 hr in 2% OsO_4 0.1M in the corresponding buffer. To 100ml 0.1M buffer, 10g sucrose were added to adjust the osmolarity for sea-water. 2) 2 hr fixation in glutaraldehyde-paraformaldehyde (1% formalin + 2.5% glutaraldehyde in 0.1M cacodylate buffer pH 7.2) and postfix for 2 hr in 2% OsO_4 in the same buffer. All fixatives were cooled to 4°C. Ethylen diamine tetra acetic acid (EDTA) was used for decalcification. After dehydration in ethanol the samples were embedded either in Epon-Araldite (Mollenhauer, 1964) or in Spurr's medium (Spurr, 1969). Semi-thin sections (0.5-1 μm) were stained with 0.1% toluidine blue solution. Sections of 70-80 nm were made on a Reichert OMU-3 microtome with glass or diamond knives, and treated for 45 min with a saturated uranyl-acetate solution (Watson, 1958) and for 5 to 8 min with a 0.4% solution of lead-citrate (Venable and Coggeshall, 1965). Sections were studied under a Zeiss EM9/S2 transmission electron microscope.

RESULTS

ANTERIOR MANTLE EDGE

GENERAL CHARACTERISTICS

The anterior pallial opening is supported by a ring of cartilage-like connective tissue as well as by muscles. It can
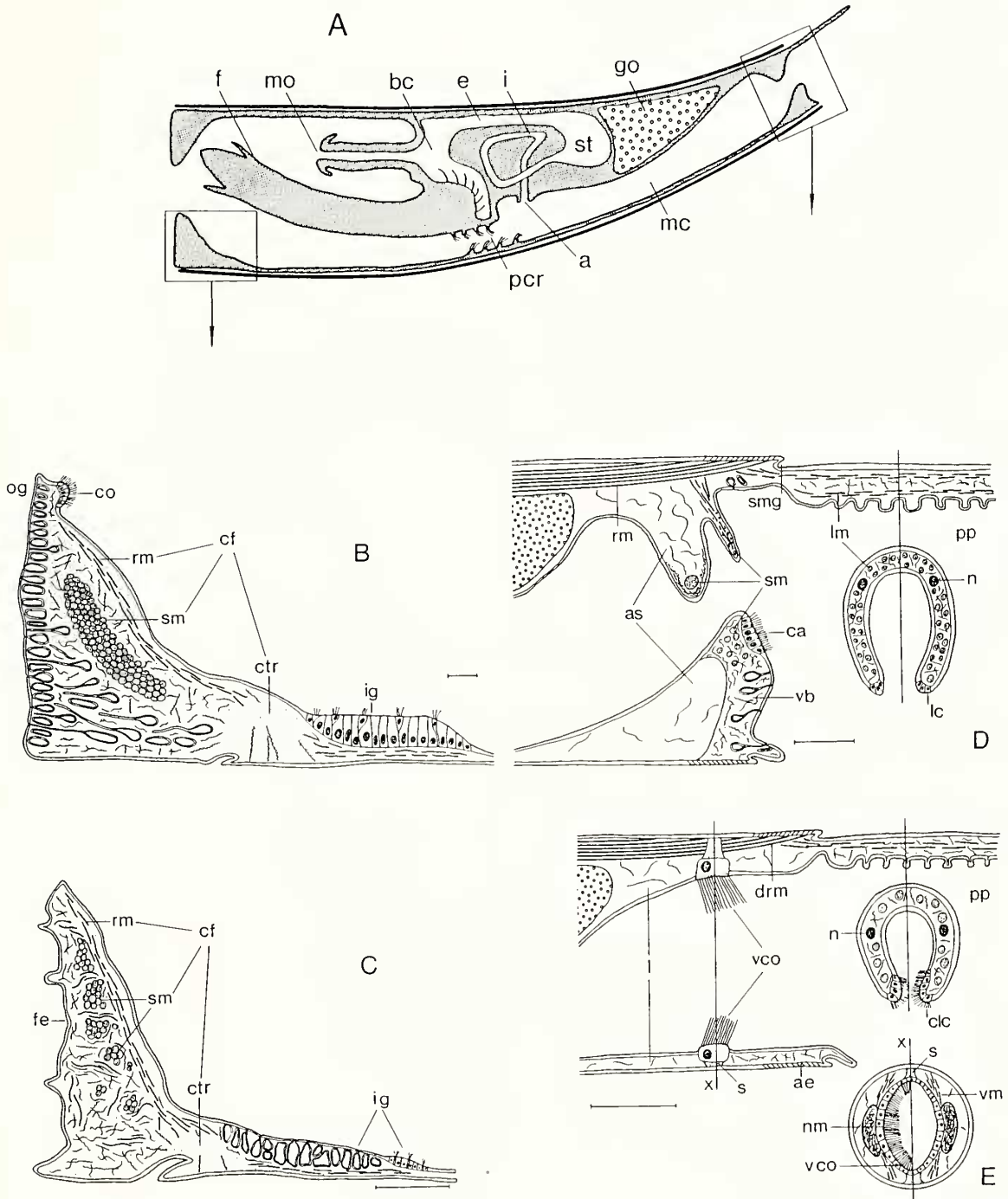
Fig. 1. A. Scaphopod in a schematic longitudinal section; B. Anterior mantle edge in the order Dentaliida (e.g. *Antalis*, semischematic longitudinal section; C. Anterior mantle edge in the order Gadilida (e.g. *Pulsellum*), semischematic longitudinal section; D. Posterior mantle edge in the order Dentaliida (e.g. *Antalis*), semischematic longitudinal section, insert (not to scale) showing cross-section of the pavillon proper; E. Posterior mantle edge in the order Gadilida (e.g. *Entalina*), semischematic longitudinal section, inserts (not to scale) showing cross-sections of the pavillon proper and the valve ciliary organ (ciliation drawn on left side only); a, anus; ae, attachment epithelium; as, annular sinus; bc, buccal cavity; ca, ciliated area; cf, central fold; clc, ciliated ledge cells; co, ciliary organ; ctr, connective tissue ring with periostracal groove; drm, dorsal retractor muscle; e, esophagus; f, foot; fe, frontal epithelium; go, gonad; i, intestine; ig, inner gland region; l, lacunae; lc, ledge cells; lm, longitudinal muscles; mc, mantle cavity; mo, mouth opening; n, nerve; nm, neuropil; og, outer gland region; pcr, preanal ciliary ridges; pp, pavillon proper; rm, radial muscles; s, socket of connective tissue; sm, sphincter muscle; smg, supramarginal groove; st, stomach; vb, ventral bolster; vco, valve ciliary organ; vm, valve muscle; x, identifies axis of cross-section in lower insert of E; scale bars = 100 μm.

exhibit different gland regions. The whole complex (see Figs. 1A-C, 2) can be divided into: 1) a frontal epithelium; 2) a central fold; 3) an inner gland region.

In all scaphopods the central fold consists of a ring of connective tissue and the muscle apparatus. The former is a wedge-shaped mass of extracellular matrix in which collagen and scaffolding fibers (fibers grillagées) are embedded (Gabe and Prenant, 1950) (Fig. 3). The orientation of the fibers is predominantly radial. The term "cartilage-like" indicates the histological and functional resemblance of this tissue to vertebrate cartilage; it does not indicate homology.

Towards the anterior aperture, collagen and muscle fibers become more abundant, replacing the extracellular matrix. Thus, the cartilage-like appearance is lost (Figs. 2, 4).

This ring provides an attachment site for the pallial muscles on both proximal and distal sides. The periostracal groove of the outer mantle epithelium has the shape of a concentric ring. Thus, constant shape and diameter are maintained by connective tissue for the shell-forming epithelia. In fixed animals the anterior mantle margin is always retracted, so the groove opens to the anterior (Figs. 1, 4). Towards the anterior aperture the central fold includes a
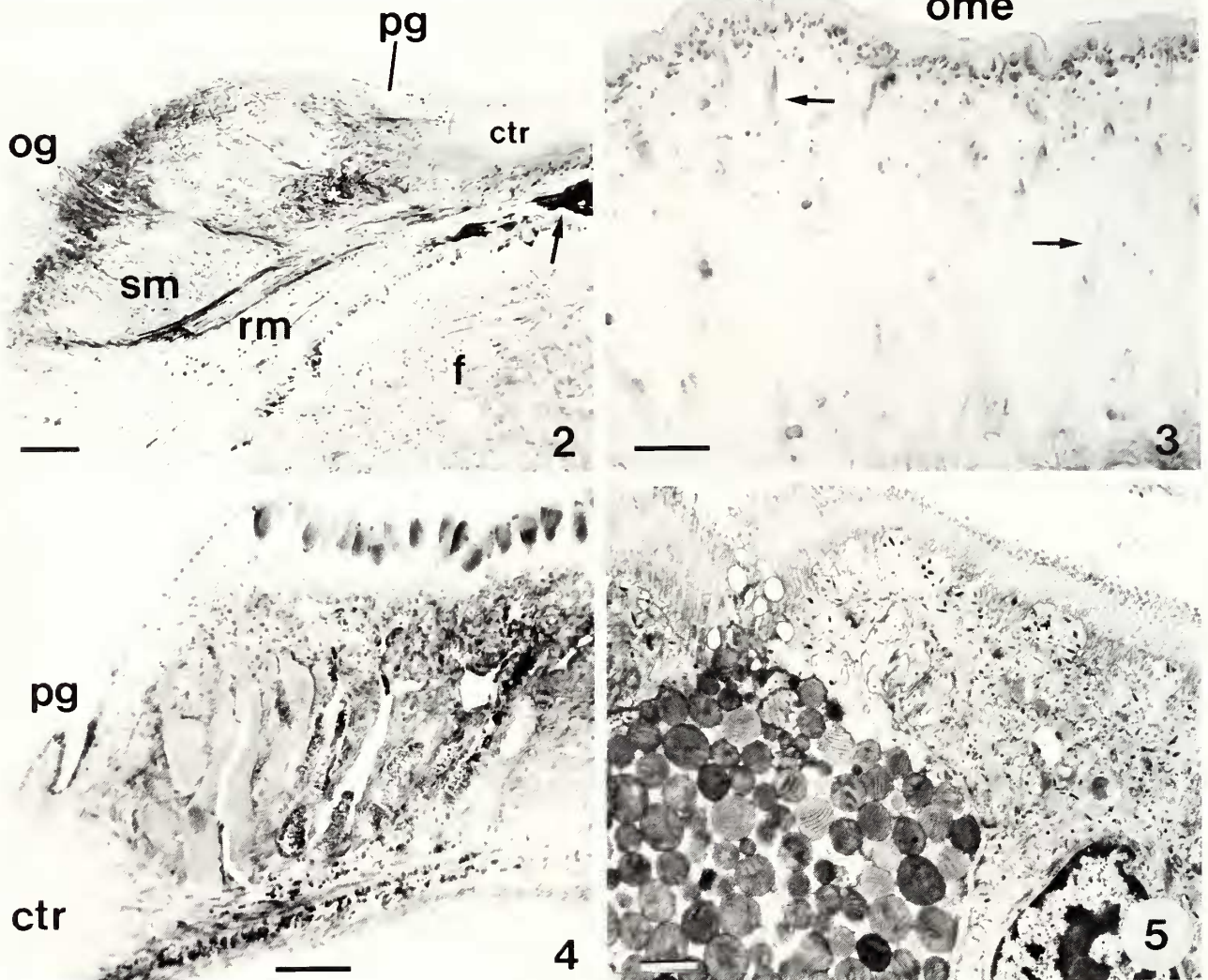

Fig. 2. *Antalis inaequicostatum*, anterior mantle edge in longitudinal section (formalin, AZAN, 7 μm), note the well developed fibrous connective tissue (asterisk); ctr, connective tissue ring; f, foot; og, outer gland region; pg, periostracal groove; rm, radial muscles; sm, sphincter muscle; inner gland region (arrow); scale bar = 100 μm. Fig. 3. *Fustiaria rubescens*, cross-section of the connective tissue ring in the anterior mantle edge (Bouin, AZAN, 7 μm); note the homogeneous intercellular matrix, where radially arranged collagen fibres (arrows) are embedded; ome, outer mantle epithelium; scale bar = 10 μm. Fig. 4. *Antalis dentalis*, longitudinal section of the outer gland region showing the different types and sizes of subepithelial gland cells (Bouin, AZAN, 7 μm); ctr, connective tissue ring; pg, periostracal groove; scale bar = 25 μm. Fig. 5. *Antalis dentalis*, TEM micrograph of the outer gland region in longitudinal section (glutaraldehyde - OsO₄, Epon-Araldite); the subepithelial gland cell contains granules of various density and with substructures; scale bar = 1 μm.

sphincter muscle that can regulate the width of the opening (Fig. 1B). Radially arranged dilator muscles function as antagonists and longitudinal fibers beneath the inner epithelium pull the fold back into the mantle cavity (Figs. 1B-C, 2). Other muscle fibers from the mantle are attached to the base of the supporting ring to retract the entire organ.

The dorsal region of the organ is innervated by a pair of cerebral nerves, the ventral portion by a pair of pleural nerves. Presence and differentiation of gland cells and sensory receptors differ within and between the orders.

DENTALIIDA

In the order Dentaliida all three components of the anterior mantle margin are developed. A detailed description representative for most dentaliid genera is given for *Antalis*, the genus with three species available for TEM studies (Table 1). *Fustiaria (= Pseudantalis) rubescens* (Deshayes); for nomenclatural considerations (see Emerson, 1951, 1952) is the only species for comparison on the ultrastructural level. Light microscopical differences between the other genera are listed in Table 2.

FRONTAL EPITHELIUM

The frontal epithelium covers the vertical surface of the anterior mantle margin facing the environment. In the Dentaliida it is identified as a glandular structure by the presence of subepithelial gland cells. It is referred to as the outer gland region. Two types of epithelial cells can be distinguished: 1) "normal" epithelial cells of 6-10 μm height with relatively electron-dense cytoplasm and a basally located nucleus; 2) cells with less dense cytoplasm and a lower density of organelles. These cells are most clearly distinguished from type 1 by foldings of the basal membrance which sometimes reach the upper quarter of the cell body. Numerous small vesicles can be seen on either side of the membrane. All cells are covered with 2-2.3 μm long microvilli and connected to each other by desmosomes and to the basal lamina by hemidesmosomes.

The subepithelial gland cells characterize the appearance of the entire dentaliid anterior mantle margin and occur in two forms (Fig. 4): barrel-shaped gland cells of 70-110 μm length can be distinguished from bottle- or flask-shaped cells that reach as far as 500 μm down into the mesenchyme of the pallial edge. The latter communicate with the surface only by long, thin ducts. Both cell types have basally located nuclei with large nucleoli. In histological sections the mucus stains from red to violet with AZAN and greenish orange with KP. Frequently the cell contents are continuous with the mucus layer covering the frontal pallial epithelium. In TEM sections the cell contents consist of spherical, membrane-bound vesicles of about 1 μm diameter. In *Antalis* spp. they show a sub-structure of straight or curved lines (Fig. 5), which is absent in *Fustiaria rubescens*. Within the same

cell, electron density of the vesicles can vary.

In longitudinal section the anterior mantle margin is somewhat triangular. In the Dentaliida the tip representing the pallial orifice carries the section of an annular ciliary organ (Figs. 1B, 6). A ring of 5-6 *(Fustiaria)* or 6-8 *(Antalis)* rows of prismatic, multiciliary cells (Fig. 7) lines the lumen of the opening, especially if the latter is contracted or closed. The nuclei are found in the proximal half of the cell, and frequently a large nucleolus is present. The cytoplasm is packed with mitochondria. Kinocilia of 15 μm length show the common 9x2+2 pattern and a basal plate. A striated rootlet (banding 56nm) is attached to the basal body at an angle of 25° to the ciliary axes. The membranes of neighboring cells are strongly interdigitated and are connected to each other by desmosomes at or close to the apex.

The ciliary organ was found in all Dentaliida examined except for *Rhabdus rectius* (Carpenter). Here, instead, two dorsolateral slits are formed by the epithelium of the distal outer gland region (Figs. 8, 9, 10A). The floor of these more or less tubular invaginations is covered by cells resembling epithelial cells, whereas the epithelium of the walls contains at least two types of specialized cells (Fig. 10B):

1) Highly columnar cells of 50 μm height with long microvilli, a nucleus close to the basal lamina, and cytoplasm that stains faint-blue with AZAN. These cells are arranged in two rows, one on each lateral wall, close to the bottom of the slit.

2) Columnar, sometimes very slender, multiciliary cells 20-30 μm high forming similar rows right above type 1. Their nuclei also lie basally, but take the red stain better than those of the first type. The cytoplasm is less dense and contains many evenly distributed dark granules. Apically, the basal bodies of the cilia appear as a line parallel to the cell membrane. These can be accompanied by gland cells that could represent a third cell type. Histologically, the outer and inner gland regions are comparable to those in the other members of the order.

RECEPTOR CELLS

The outer gland region and the epithelium of the central fold are marked by the presence of ciliary sensory receptors. In the three species of *Antalis* ultrastructurally examined, two types can be distinguished.

Type 1 (Figs. 11A, 12, 13), very abundant in *Antalis* spp., represents a swollen process of a nerve cell. This sensory cell is more or less cylindrical with a maximum diameter between 2.4 μm and 3.8 μm. No nucleus was found. It is, therefore, thought to lie in the perikaryon of the nerve cells in the underlying mesenchyme. The dendritic processes were traced only for short distances beneath the epidermis. The cytoplasm appears electron lucent except for scattered neurotubules. Close to the epithelial surface several large, elongated mitochondria can be found. They adjoin the
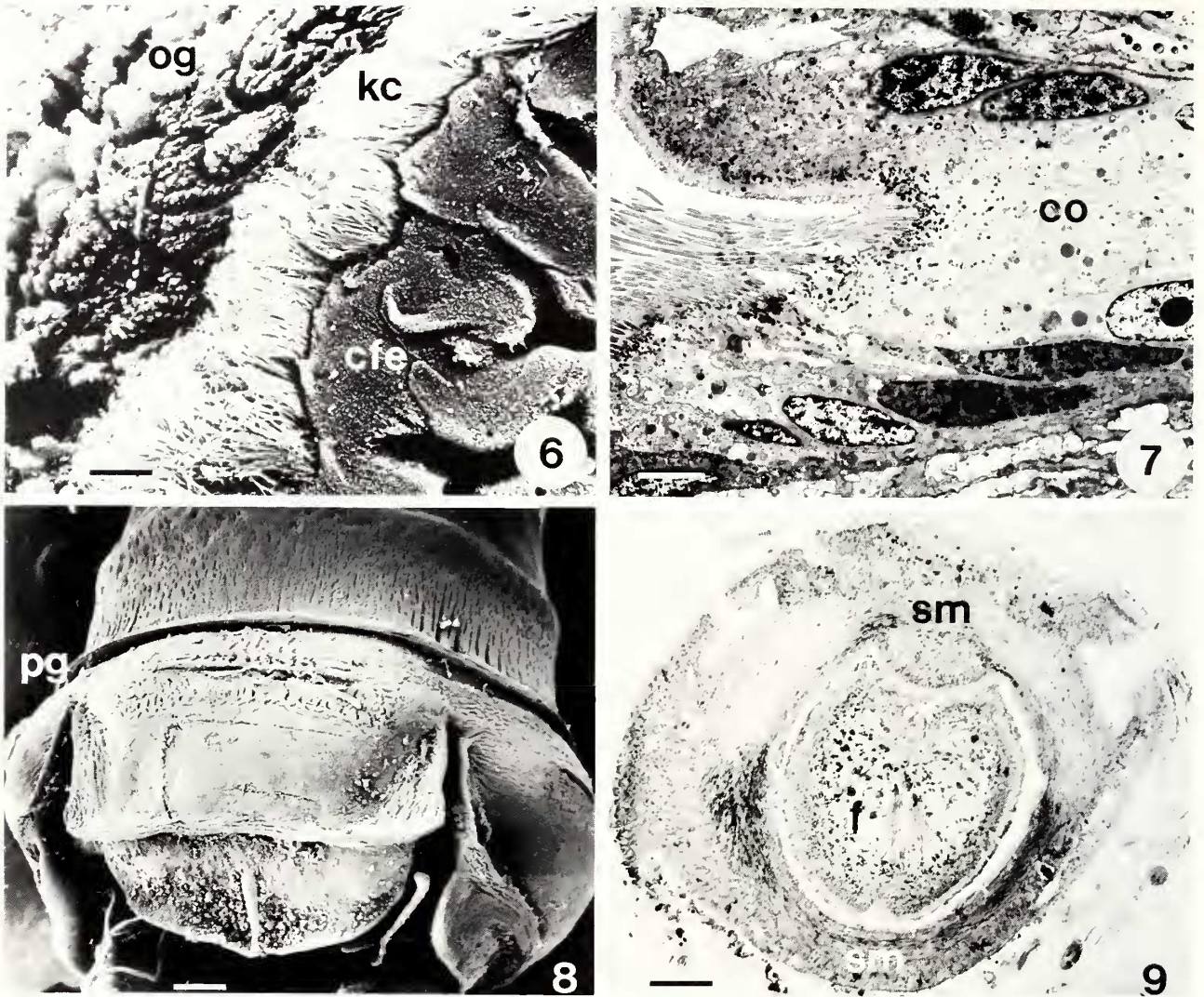
Fig. 6. *Fustiaria rubescens*, SEM micrograph of the anterior mantle edge showing the ciliary organ; cfe, central fold epithelium; kc, kino-cilia; og, outer gland region; scale bar = 5 μm. **Fig. 7.** *Antalis occidentalis*, TEM micrograph of the ciliary organ in cross-section (glutaraldehyde - OsO₄, Epon-Araldite); note the abundance of dark granules close to the cell apices; co, cells of the ciliary organ; scale bar = 5 μm. **Fig. 8.** *Rhabdus rectius*, SEM micrograph of the anterior mantle edge in dorso-frontal view; note the slit-like invaginations; pg, periostracal groove; scale bar = 300 μm. **Fig. 9.** *Rhabdus rectius*, cross-section of the anterior mantle edge with the dorso-lateral slits (formalin, AZAN, 7 μm), f, foot; sm, sphincter muscle; scale bar = 250 μm.

centrioles of the sensory cilia. The number of cilia varies from 5 to 25, but the higher number is more common. With a length of 2-4 μm, the cilia are shorter than those of the ciliated epithelial cells of the central fold. The bases of the receptors are slightly sunken in, although the cell apices can protrude over the other epithelial cells. Some scanning electron microscographs reveal tapering cilia, suggesting reduction of pairs of microtubules towards the tip. Connections to neighboring cells are effected by desmosomes and septate junctions.

Type 2 represents a collar-receptor (Figs. 11C, 14, 15), which is much less abundant in *Antalis* spp. than in *Fustiaria rubescens*. The dendritic process is more slender than that of type 1, measuring 1-2 μm across. One or two collar struc-

tures have been observed for one sensory cell. One 5-6 μm long cilium is surrounded by 8 modified microvilli, so-called stereomicrovilli (Haszprunar, 1985; Salvini-Plawen, 1988), which are basally connected by a membrane. Many filaments can be observed within the stereomicrovilli, especially in the region adjacent to the cilium.

In *Fustiaria rubescens*, type 1 is rare and predominantly found in the outer gland region. Type 2, the collar-receptor, is more common and found mainly in the epithelium of the central fold. Both receptor types are similar in all aspects to the respective types in *Antalis* spp. Another kind of receptor cell, here termed type 1a (Figs. 11B, 16), appears in both epithelial regions. It has a diameter of about 1.5 to 2 μm and the cytoplasm is packed with ellipsoidal
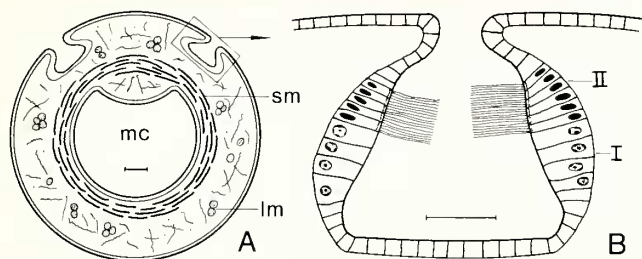
Fig. 10. *Rhabdus rectius.* **A.** semischematic cross-section of the anterior mantle edge; scale bar = 300 μm; **B.** detail from A. showing the epithelium of the dorso-lateral slit; scale bar = 200 μm.

mitochondria (0.4 x 0.6 μm). Apically, 4-5 cilia of normal structure can be seen. A tiny rudiment of a rootlet inserts at the single centriole.

INNER GLAND REGION

The inner gland region, a differentiation of the inner pallial epithelium, terminates the complex of the anterior mantle edge proximal to the central fold. It forms a tube of epithelial, mucus-secreting cells of variable extent, probably depending on the state of contraction of the underlying mantle muscles. The barrel-shaped epithelial gland cells (Fig. 17), measuring approximately 40-90x20x20 μm, are filled with densely packed vesicles of light- and electron lucent appearance, which have the tendency to coalesce to a single droplet of mucus. The nucleus is generally located close to the thin basal lamina. Another type of gland cells, restricted to few specimens of *Antalis* species only, is flask-like with

a larger basal portion and a thin process passing between the other cells to reach the surface (Fig. 2). Their contents vary from violet to bright red with AZAN and greenish orange in KP. At present, it is not clear whether these cells are epithelial or subepithelial. Apart from these, no subepithelial gland cells were recognized in the inner gland region of Dentaliida. The surface of the gland cells is partly covered by ciliated epithelial cells with apically located nuclei (Fig. 17). These cells are attached to the basal lamina by very slender processes only.

GADILIDA

The anterior pallial edge in Gadilida has the same basic structure as in dentaliids, but the entire organ has undergone different elaboration (Fig. 1C). The ring of cartilage-like connective tissue is generally not as extensive as in the Dentaliida. In some species only a narrow band at the base of the periostracal groove is differentiated. Plate (1892) and Odhner (1931) noted the absence of the outer gland region in members of this order. The ciliary organ of the central fold is absent.

FRONTAL EPITHELIUM

Characteristic for the gadilid frontal epithelium is the elaboration of papillae. Their number is high in the representatives examined of the families Entalinidae, Pulsellidae and Wemersoniellidae. In Siphonodentaliidae and Gadilidae, however, papillae are more scattered. In *Entalina quinquangularis* (Forbes), papillae are 15 and 20 μm high. Depending on the state of contraction the conical protrusions
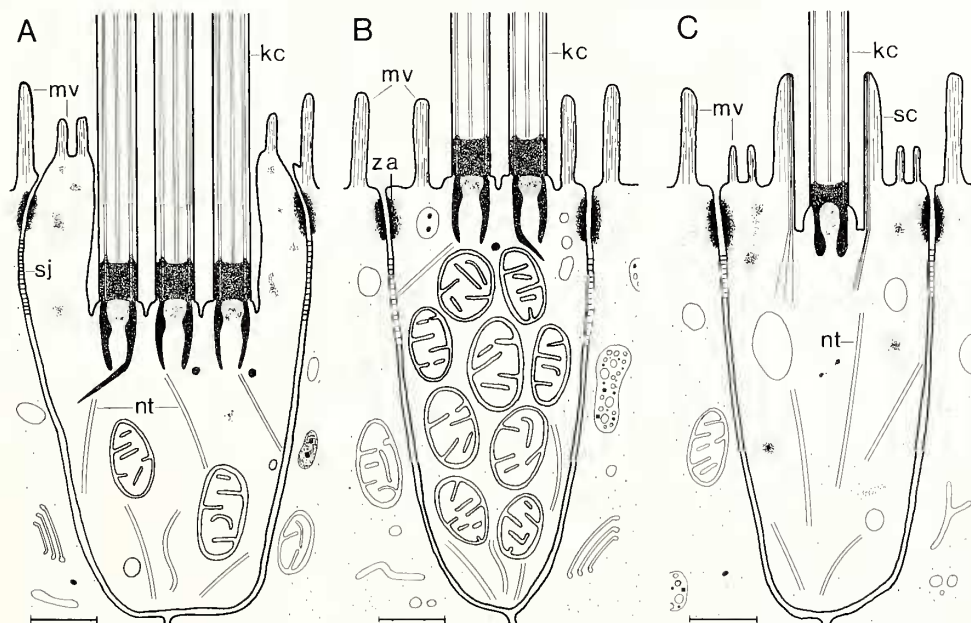

Fig. 11. Reconstruction of ciliary sensory cells of the anterior mantle edges. **A.** Type 1 of *Antalis;* **B.** Type 1a of *Fustiaria;* **C.** Type 2 (collar receptor) of *Antalis;* kc, kinocilium; mv, microvilli; nt, neurotubules; sj, septate junction; za, zonula adherens; scale bars = 0.5 μm.
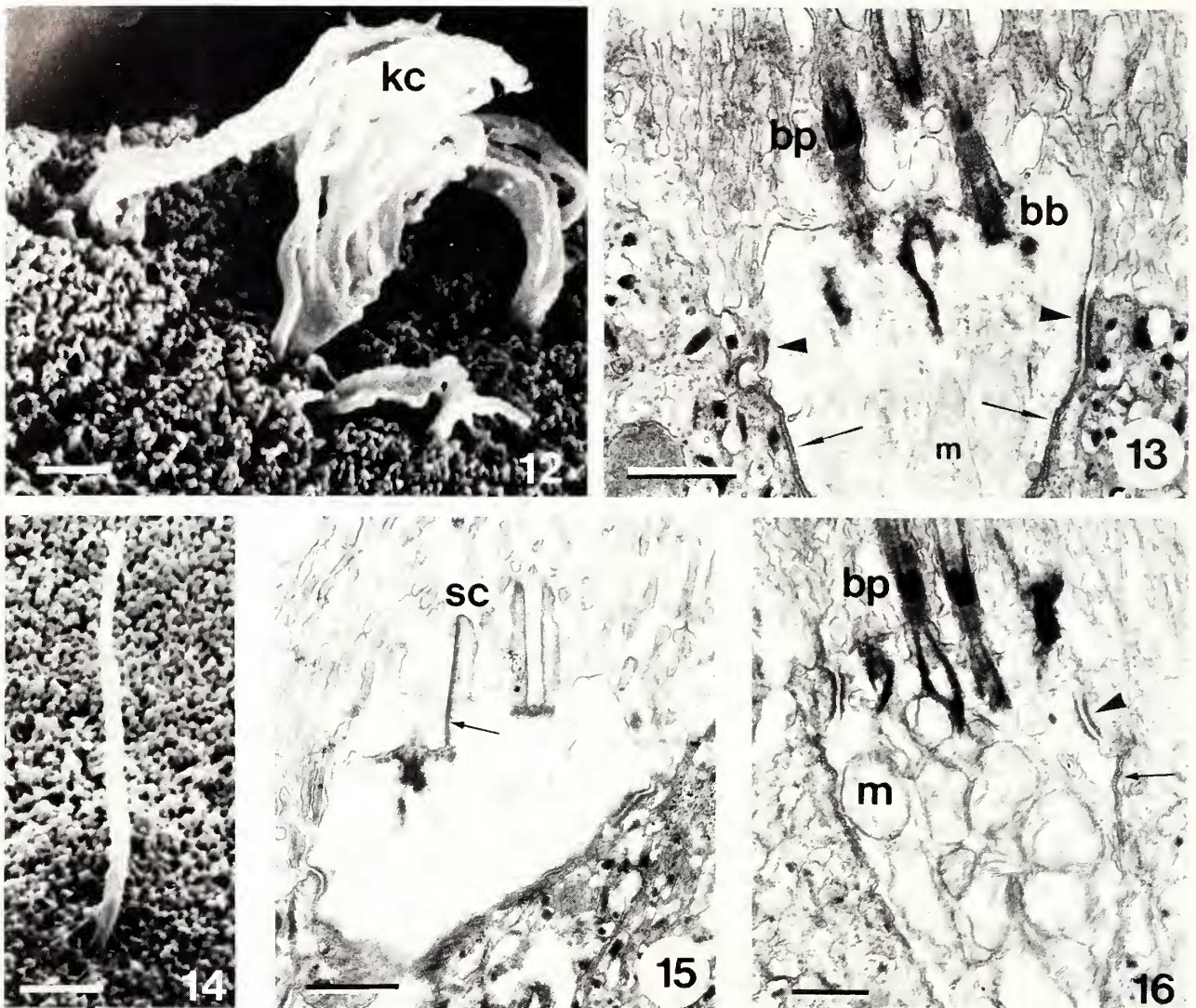
Fig. 12. *Antalis occidentalis,* SEM micrograph of a ciliary sensory cell of type 1 in the frontal epithelium of the anterior mantle edge; kc, kinocilia; scale bar = 0.5 μm. Fig. 13. *Antalis occidentalis,* TEM micrograph of a type 1 receptor in a longitudinal section (glutar-paraformaldehyde - OsO₄, Spurr); bb, basal body; bp, basal plate; m, mitochondrion; septate junctions (arrows), zonulae adherentes (arrow-heads), scale bar = 0.5 μm. Fig. 14. *Antalis occidentalis,* SEM micrograph of a type 2 receptor (collar receptor) of the frontal epithelium; scale bar = 0.5 μm. Fig. 15. *Antalis dentalis,* TEM micrograph of a type 2 receptor in a longitudinal section (glutaraldehyde - OsO₄, Epon-Araldite); note the two collar structures; sc, stereocilia (=stereomicrovilli); dense microfilaments in a stereocilium (arrow-head), scale bar = 0.5 μm. Fig. 16. *Fustiaria rubescens,* TEM micrograph of a type 1a receptor cell in the frontal epithelium (glutaraldehyde - OsO₄, Epon-Araldite); bp, basal plate; m, mitochondrion; septate junction (arrows); zonula adherens (arrow-head); scale bar = 0.5 μm.

are either approximately 30 μm apart, so they are elaborated on a flat base of tissue close to each other (Figs. 18, 19). Generally, papilla density is highest close to the mantle opening and decreases towards the periphery.

The transition from the frontal epithelium to the outer mantle surface is marked by a crest of connective tissue (Figs. 18, 19). The periostracal groove is visible some distance below the crest on the outside of the mantle. In fixed specimens the frontal epithelium is sunken in, giving the mantle margin the appearance of a crater rimmed by the above-mentioned crest.

The frontal epithelium cells are rather flat - 3 μm in *Cadulus subfusiformis* (M. Sars), 5 μm in both *Entalina quinquangularis (Forbes)* and *Pulsellum lofotense* (M. Sars). The latter also possess vesicle containing cells which appear to be epithelial gland cells.

An unsual tissue is located beneath the frontal epithelium of *Entalina quinquangularis.* Being only about 15 μm thick, this layer is poorly visible in histological sections (Fig. 19, x). The tissue consists of spherical, vacuolized cells that proved to be problematic in TEM preparations, because their contents, perhaps of mucoid nature, were washed out.
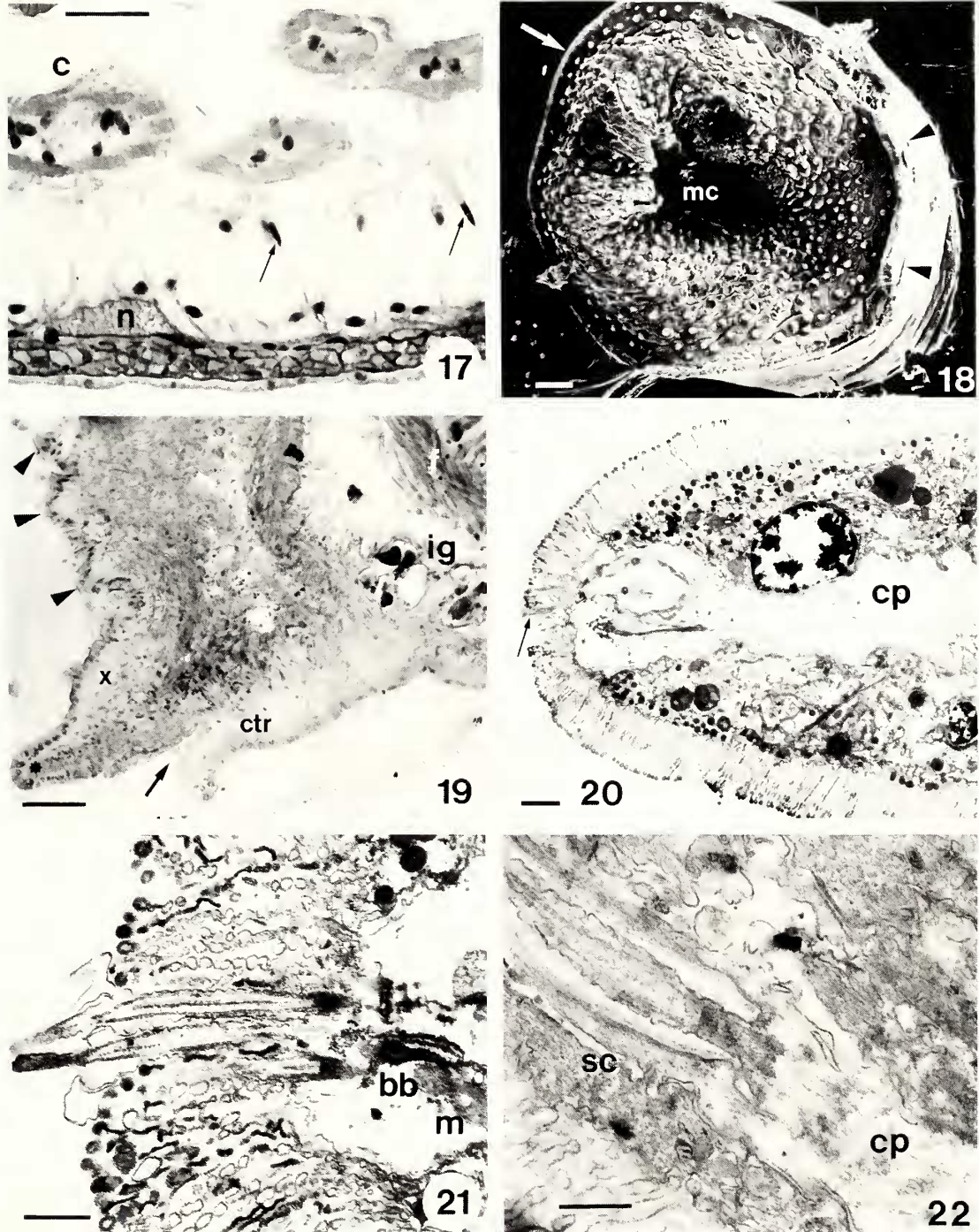
Fig. 17. *Fustiaria rubescens,* cross-section of the inner gland region (Bouin, AZAN, 7 μm) showing epithelial gland cells; note the position of the nuclei of the ciliated epithelial cells (arrows); c, captaculum; n, pallial nerve; scale bar = 25 μm. **Fig. 18.** *Entalina quinquangularis,* frontal view of the anterior mantle edge in a SEM micrograph (glutaraldehyde - OsO$_4$, Spurr); note the numerous papillae; mc, opening of the mantle cavity; periostracal groove (arrowheads); rim of the "crater" (arrow); scale bar = 100 μm. **Fig. 19.** *Entalina quinquangularis,* anterior mantle edge in longitudinal section (Bouin, AZAN, 7 μm); note the less voluminous ring of turgid connective tissue (ctr); f, foot, ig, inner gland region; x, mucoid tissue layer; papillae (arrow-heads); periostracal groove (arrow), rim of the "crater" (asterisk); scale bar = 100 μm. **Fig. 20.** *Entalina quinquangularis,* TEM micrograph of a frontal epithelial papilla in longitudinal section (glutaraldehyde - OsO$_4$, Spurr); cp, sensory cell process; cilium of the apical sensory cell (arrow), scale bar = 1 μm. **Fig. 21.** *Entalina quinquangularis,* ciliary sensory cell at the tip of a papilla (glutaraldehyde - OsO$_4$, Spurr), bb, basal body; m, mitochondria; scale bar = 0.5 μm. **Fig. 22.** *Cadulus subfusiformis,* pitted ciliary sensory cell (type 2, collar-receptor) at the tip of a papilla (glutarparaformaldehyde - OsO$_4$, Spurr), cp, sensory cell process with neurotubuli, sc, stereocilium (=stereomicrovillus); scale bar = 0.5 μm.

Delicate fibers of connective and muscle tissue fill the intercellular spaces. For other members of the family Entalinidae, available from museum samples only, this tissue could not conclusively be found.

RECEPTOR CELLS

Although some type 1 receptor cells were found in the frontal epithelium of *Cadulus subfusiformis*, the sensory elements of the gadilid anterior mantle margin are found in the papillae. Each papilla carries one or more sensory processes of neurons (Fig. 20), whose perikarya lie in the connective tissue beneath. A connection between a receptive process and such a perikaryon was not found. Nor was it possible to trace the process of the sensory cell beneath the base of the papilla. The cytoplasm appears optically empty and only neurotubles can be seen orientated mainly in the direction of the cell axis. Two or more cilia (9x2+2 pattern) protrude from the apex (Fig. 21). A basal plate and a single centriole form the anchoring apparatus. No rootlet is developed, but small bundles of fibers spread from the centriole to the surrounding mitochondria (Fig. 21). The mitochondria are elongted and measure about 1x0.4 μm. Some similarity to the type 1 receptors of dentaliids is evident. Papillae with collar-receptors appear in *Cadulus subfusiformis* (Fig. 22).

INNER GLAND REGION

The inner gland region in Gadilida is mainly formed by subepithelial gland cells. Accordingly, the cell bodies, apart from the secretory duct, are located beneath the basal lamina. Like other areas of the mantle cavity the inner gland region is covered by a cuboidal epithelium. The form of the gland cells, influenced by the packing between the basal lamina and the pallial muscles, is cuboidal to honeycomb-like. Frequently, they over- and underlay each other (Figs. 23, 24).

Staining with AZAN reveals two types of subepithelial secretory cells: one staining violet to red, the other taking no stain (Fig. 23). In TEM-sections both types contain small vesicles that show tendencies to coalesce to larger secretion vacuoles. A cell can be completely filled with a single, membrane-bound droplet (Fig. 24). Electron density seems to parallel the histological staining properties. The nucleus with a large nucleolus usually lies near the base of the cell. The cytoplasm surrounding the nucleus is rich in golgi complexes.

The proximal border of the gland region is formed by a few rows of epithelial gland cells. They are prismatic in shape and stain in a faint blue or remain unstained with AZAN. Unfortunately, this tissue is easily damaged by improper fixation, processing, or long storage; therefore, it is usually not preserved in museum material. Table 2 lists the characters of the anterior mantle margin for the examined genera.

EPITHELIUM OF THE
MANTLE CAVITY

Most of the pallial cavity is lined by flat epithelial cells.

Table 2. Characters of the anterior mantle margin: (++) abundant; (+) present; (±) scarce, few; (−) absent; (?) no information.

	Frontal Epithelium				Ciliary Organ		Receptor Cell Types				Inner Gland Region		
	subepith. gland cells	epith. gland cells	papillae	"mucoid" tissue	cell rows	slits	1	1a	2	papil- lae	subepith. gland cells	mucoid epith. gland cells	dark epith. gland cells
Antalis	++	−	−	−	6-8	−	+	−	±	−	−	+	+
Dentalium	++	−	−	−	6-8	−	?	?	?	−	−	+	−
Fissidentalium	++	−	−	−	6-8	−	?	?	?	−	−	+	−
Graptacme	++	−	−	−	?	−	?	?	?	−	−	+	−
Fustiaria	++	−	−	−	5-6	−	±	+	+	−	−	+	−
Laevidentalium	++	−	−	−	+	−	?	?	?	−	−	+	−
Rhabdus	++	−	−	−	−	+	?	?	?	−	−	+	−
Entalina	−	±	++	+	−	−	−	−	−	+	+	+	−
Bathoxiphus	−	?	++	?	−	−	?	?	?	?	+	+	−
Heteroschismoides	−	?	++	?	−	−	?	?	?	?	+	+	−
Pulsellum	−	±	++	−	−	−	−	−	−	+	+	+	−
Annulipulsellum	−	?	++	−	−	−	?	?	?	?	+	+	−
Striopulsellum	−	?	++	−	−	−	?	?	?	?	+	+	−
Siphonodentalium	−	?	±	−	−	−	?	?	?	?	+	+	−
Wemersoniella	−	?	++	−	−	−	?	?	?	?	+	+	−
Gadila	−	?	±	−	−	−	?	?	?	?	+	+	−
Cadulus	−	−	±	−	−	−	+	−	−	+	+	+	−

The mantle area anterior to the head is characterized by the muscle fibers embedded between the outer and the inner mantle layer. The fibers emerge from the dorsal body retractors. Posterior to the head the musculature decreases conspicuously. Just in front of the cerebral ganglia the two layers separate: the outer layer continues into the dorsal body epithelium, the inner one meets the epidermis of the head. The line of mantle attachment descends posteriorly to a level between the equator and the upper third of the more or less cylindrical body. Behind the muscular part of the mantle the tubules of the digestive gland and, especially in Gadilida, the gonads are spread laterally between the mantle layers.

Lacaze-Duthiers (1856-7) first described numerous transverse ciliated ridges along the anal region. In fact, these ridges are epithelial rings running around the entire pallial cavity including the body wall. Each ring consists of one row of cells (Figs. 25, 26). In dentaliids the number of rings ranges from 5 in some *Antalis dentalis* specimens to 22 in *Rhabdus rectius* . The average number is between 12 and 15. In the order Gadilida usually four to eight ciliary rings are present. In *Gadila metivieri* Scarabino, however, only one ring could be distinguished, whereas a specimen of *Siphonodentalium vitreum* M. Sars had 15 rings. The number of ridges is probably body-size dependent. The distance between two rows ranges between 10 μm and 40 μm, depending on the state of contraction of the mantle tissue. In some specimens of various species mucus secreting cells could be identified between the ridges. In *Cadulus aberrans* Whiteaves, three to four spherical gland cells with mucoid contents occur in each interval (Fig. 26).
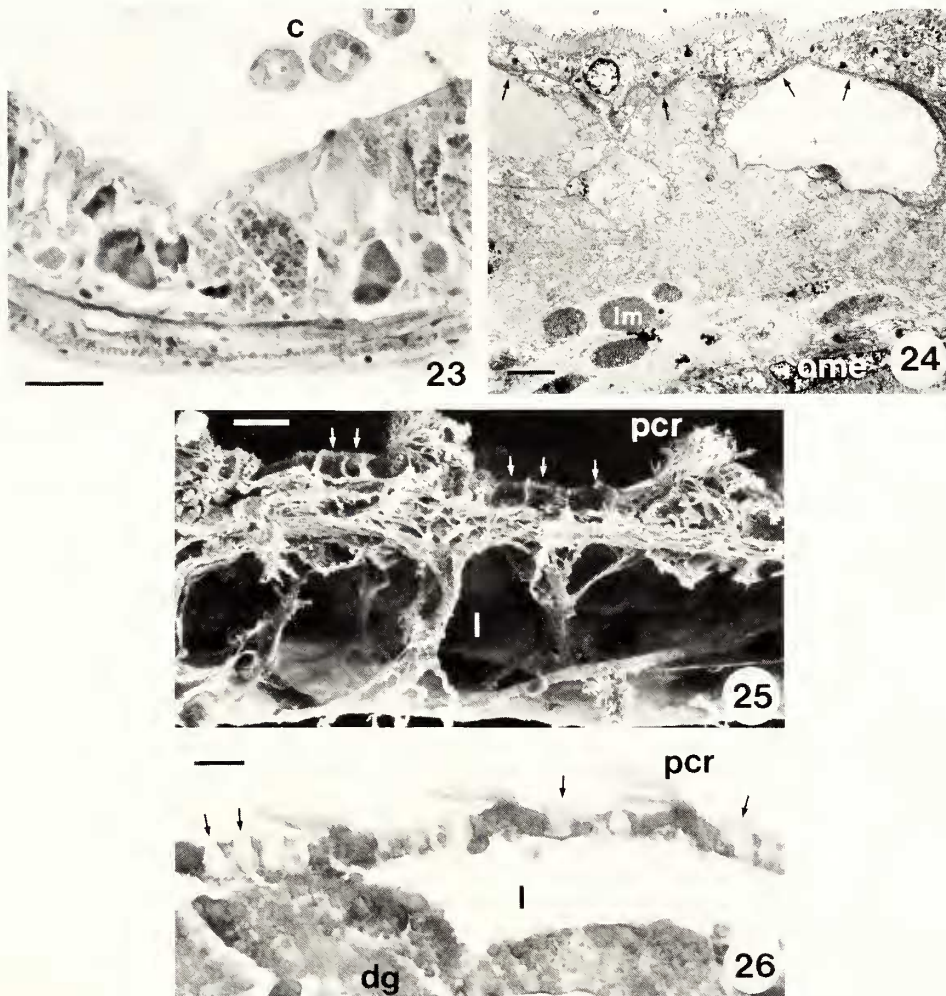

Fig. 23. *Entalina quinquangularis*, longitudinal section (Bouin, AZAN, 7 μm) of the inner gland region, showing subepithelial gland cells; c, captaculum; scale bar = 25 μm. **Fig. 24.** *Entalina quinquangularis*, TEM micrograph of the inner gland region in cross-section (glutaraldehyde - OsO$_4$, Epon-Araldite); note the subepithelial gland cells in various stages beneath the basal lamina (arrows); lm, longitudinal pallial muscles; ome, outer mantle epithelium; scale bar = 3 μm. **Fig. 25.** *Antalis dentalis*, SEM micrograph of a longitudinal section of the mantle in the preanal region; l, lacunae; pcr, preanal ciliary ridge; note the epithelial gland cells between the ridges (arrows); scale bar = 10 μm. **Fig. 26.** *Cadulus subfusiformis*, longitudinal section of the same region (glutaraldehyde, AZAN, 7 μm); dg, digestive gland tubule; l, lacunae; pcr, preanal ciliary ridge; epithelial gland cells between the ridges (arrows); scale bar = 5 μm.

In both orders the cilia-bearing cells (30-40 μm wide and 15-20 μm high) protrude conspicuously from the cuboidal mantle epithelium. The densely granulated cytoplasm stains from steel-blue to dark red with AZAN, red with HE and light brown with KP. The nucleus usually lies eccentrically, close to the posterior cell wall. The centrioles form conspicuous lines of strongly refractive granules below the apical cell membrane. Cilia length is approximately twice the height of the cell. In most of the sections the cilia point posteriorly. No ultrastructural work had been done on this mantle region.

Most of the Dentaliida and some Gadilida have a glandular area anterior to the ciliated ridges. Distaso (1906) homologized this area with the hypobranchial gland of other molluscs. The cells resemble those of the inner gland region, albeit much higher (80 μm). These delicate and vulnerable cells easily disintegrate when treated improperly. Thus, no clear evidence is drawn from the analysis of this character.

Spacious blood lacunae are located between the mantle layers below the ciliar ridges (Figs. 25, 26). Towards the posterior mantle region they unite to a single sinus along the ventral midline. The epithelium of the pallial cavity above this blood sinus bulges out.

POSTERIOR MANTLE EDGE

The posterior mantle edge, or pavillon (according to Deshayes, 1825 and Lacaze-Duthiers, 1856-7), is situated at the apical aperture of the shell. Inhalent and, partly at least, exhalent currents pass through this opening. In both scaphopod orders the dorsal retractor muscles have a single ring-shaped attachment site (Fig. 27) on the inner shell surface a short distance from the apex. The pallial orifice represents a valve apparatus to regulate the water flow. The structure and function of this valve-organ differ within Dentaliida and between Dentaliida and Gadilida. Distal to this organ, a dorsal process of the pallial epithelium, the pavillon proper, is differentiated. Different types of gland cells can be distinguished.

The following descriptions are based mainly on histological observations. The situation in the examined species of *Antalis* is representative for all other dentaliid species studied except *Fustiaria* spp. The posterior mantle margin of *Entalina quinquangularis* is described for the Gadilida. The other species of this order do not differ substantially. An interpretaion of the functional morphology is provided in the discussion.

ANTALIS

Although Leon (1895) and Boissevain (1904) published descriptions of the histological structure of the pavillon of *Antalis vulgaris* (DaCosta), this organ will be redescribed here. Close to the tip of the shell, where the retractor muscles attach, a ring-like protrusion of the mantle tissue terminates
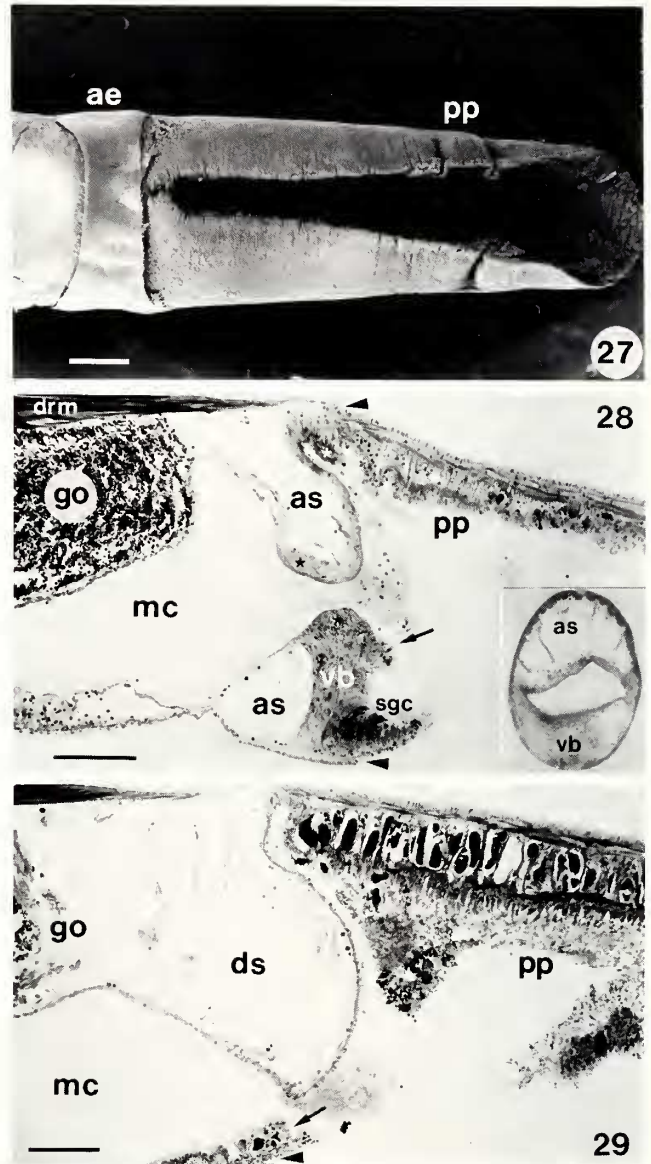

Fig. 27. *Fissidentalium candidum*, SEM micrograph of the posterior mantle edge and the pavillon proper in ventral view; ae, attachment epithelium; pp, pavillon proper; scale bar = 1 mm. Fig. 28. *Antalis inaequicostatum*, posterior mantle edge in longitudinal section (Bouin, AZAN, 7 μm); insert (not to scale) shows cross-section; as, annular sinus; drm, dorsal retractor muscle; go, gonad (testis); mc, mantle cavity; pp, pavillon proper; sgc, subepithelial gland cells; vb, ventral bolster; ciliated area (arrow); supramarginal groove (arrow-heads); sphincter muscle (asterisk); scale bar = 100 μm. Fig. 29. *Fustiaria rubescens*, posterior mantle edge in longitudinal section (Bouin, AZAN, 7 μm); note the high abundance of subepithelial gland cells in the pavillon proper; ds, dorsal sinus; go, gonad; mc, mantle cavity; pp, pavillon proper; ciliated area (arrow); supramarginal groove (arrow-heads); scale bar = 100 μm.

the pallial cavity. The base of this annular fold is formed by a huge annular blood sinus, which connects the haemolymph spaces of the gonad region with the mid-ventral lacuna of the mantle (Figs. 1D, 28). The dorsal part of the fold is produced

entirely by this sinus. On the ventral side, however, the sinus is underlain posteriorly by a crest of connective tissue, the ventral bolster. In histological sections this tissue resembles the fibrous connective tissue of the anterior mantle edge, showing scaffolding and high densities of collagen fibers (Fig. 28). These fibers make the tissue look denser than the anterior counterpart. Although this tissue is voluminous at the bottom of the annular fold, it rapidly decreases laterally and towards the dorsal side. In the blood sinus it is present only as a narrow layer beneath the epithelium. Another branch of this tissue is part of a smaller, distal projection of the pallial wall, previously undescribed (Fig. 28). Several muscle strands extend through the dorsal section of the sinus (Figs. 1D, 28). They originate from the dorsal retractors and insert on the layer of connective tissue. Therefore, the main direction of the fibers is from anterior-dorsal to posterior-ventral. When contracting, these muscles withdraw the upper part of the annular fold and increase the diameter of the opening. The antagonistic force is provided by a bipartite sphincter muscle. It has two dorsal portions that unite ventrally to a single mass. The anterior dorsal portion within the blood sinus appears as a compact, naked bundle closely joined to the supporting tissue of the epithelium (Figs. 1D, 28). The posterior dorsal portion, together with a layer of connective tissue, forms a narrow but rigid semi-annular projection. On the ventral side, the fibers radiate into the ventral bolster. In the ventral section of the sinus only a few, more ligament-like fibers are present.

Subepithelial gland cells are located in the ventral bolsters. They open towards the pavillon on the posteriorly facing wall of the valve apparatus (Fig. 28). These unicellular glands have a red staining, granulated cytoplasm that resembles the dark staining cells of the outer gland region in the anterior mantle edge (Leon, 1895; Boissevain, 1904). They extend about 25 μm deep into the connective tissue. In some sections the second, weakly staining cell type can also be found, although this type is much less abundant than the other. Sometimes, slightly different cells are found in the dorsal region. Their secretory granules are larger and stain bright red. This type is also present in the pavillon proper. An area of ciliated cells is located either on the outer base or on the outer flank of the valve (Boissevain, 1904; Reynolds, 1988). Reynolds (1988) described two type of ciliary receptors in this region. The rest of the epithelium of the ring-fold consists of low-prismatic cells with large nuclei.

The attachment epithelium of the retractor muscles is followed posteriorly by a shallow supramarginal groove (Stasek and McWilliams, 1973), which separates the valve mechanism from the pavillon proper (Figs. 28, 29). This is a dorsally elongated pallial appendage resembling the tip of a syringe needle (Fig. 27). Thus, the opening has an oblique plane facing the ventral side. In cross section the pavillon proper is crescent-shpaed. The inner epithelium of the

pavillon is extensively folded, probably a consequence of the contracted longitudinal muscles of this organ. In the cytoplasm, numerous small granules stain a brownish-green color with AZAN. The large nucleus stains red. Subepithelial gland cells with red granules, belonging to one of the two mentioned types, occur in longer but regular intervals. The ledges of the crescent are covered by cells of a different type. They are more columnar, showing dark-red cytoplasm and large, elongated nuclei. Their function is unclear. Scanning electron micrographs (unpublished) showing tufts of cilia indicate a sensory function. The outside of the pavillon is covered by an epithelium of cuboid cells, which is usually not folded like the inner one. No glands or gland openings could be recognized there.

A loose matrix of connective tissue can be observed between the epidermal layers of the pavillon. Muscles, nerves and the gland cells of the inner surface are embedded here. Conspicuous longitudinal muscle fibers insert at the dorsal side of the attachment epithelium. These shorten the pavillon when contracted. A web of more delicate transverse and dorsoventral muscles are responsible for extension. The entire structure is highly motile, although its movements are usually slow. Haemolymph pressure is unlikely to play an important role in pavillon extension because only a minor mid-dorsal lacuna enters from the annular sinus. After a short distance, this blood space branches into numerous small lacunae.

Innervation of this mantle appendage is achieved by a pair of large nerves. Their origin lies in a pair of small ganglia at the base of the pavillon, which receive connectives from the visceral ganglia. The nerves usually extend and wind through the pavillon. Towards the tip of the pavillon they taper in diameter, but are always clearly detectable.

FUSTIARIA

Both species of *Fustiaria* examined show important differences from *Antalis* spp. and other Dentaliida examined in the structure of the posterior mantle edge, particularly with respect to the valve mechanism and the glandular components of the pavillon order (Figs. 29, 30).

The valve is a simple elaboration of a large dorsal blood sinus. It bulges ventrally and thus forms a lid for the mantle pore. No annular blood sinus, sphincter muscle, or ventral bolster of connective tissue are present in specimens of this genus. The large blood space extends from the tip of the gonad to the muscle attachment site. Similar to *Antalis* spp., it is transversed by many small muscle fibers. Their origin is the dorsal body wall and they insert on the thick basal lamina of the ventral and lateral epithelium or the layer of connective tissue beneath. Ventrally, the mantle cavity ends with a slightly thickened rim just behind the attachment epithelium. The rim contains some circular and dorsoventral muscle fibers and connective tissue, which is not comparable
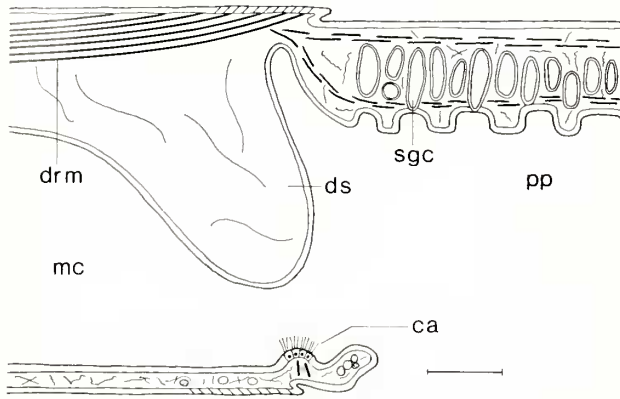
Fig. 30. *Fustiaria rubescens,* semischematic longitudinal section of the posterior mantle edge; note the lacking of the ventral bolster and the high abundance of subepithelial gland cells in the pavillon proper; ca, ciliated area; ds, dorsal sinus; drm, dorsal retractor muscles; mc, mantle cavity; pp, pavillon proper; sgc, subepithelial gland cells; scale bar = 100 μm.

to the differentiations in *Antalis* spp. Longitudinal sections reveal some ciliated cells belonging to a weakly defined ciliated area of the ventral mantle epithelium. This is probably the equivalent of the more clearly defined area of *Antalis* spp.

With the lack of the ventral bolster, the incorporated gland cells are likewise missing, but the rich development of secretory elements in the inner epithelium of the pavillon proper (Figs. 29, 30) compensates for this. Large gland cells fill the space between the longitudinal muscles of the pavillon throughout the entire length. With AZAN the granules stain from bright red to dark blue and purple (Fig. 29). It is difficult to determine whether they represent different cell types or subsequent physiological stages.

ENTALINA

The apical pallial orifice in the Gadilida can be closed to a vertical slit by a pair of septa arising laterally in the pallial cavity. The slit is closed by the contraction of dorsoventrally orientated muscles lying beneath and on either side of the basal lamina of the epithelium. These muscles, joining in the dorsal and ventral mid-line, insert upon common sockets of connective tissue. Nerves from the small pavillon ganglia descend along the muscle bundles (Figs. 1E, 31). Radial muscle bundles connect inner and outer mantle epithelia.

The epithelium of the slit differentiates a conspicuous ciliary organ (Figs. 1E, 32). It consists of 25 (±2) large, densely ciliated cells arranged in a single row encircling the lumen of the mantle opening. These cells are about 12 μm high and 25-30 μm wide. The large spherical nuclei, often with obvious nucleoli, are located centrally in the cytoplasm. The cytoplasm is packed with mitochondria. Numerous vesicles of different diameters and electron densities are present. Small electron-dense granules can be seen close to the cell apex. The cell surface is covered with tightly packed cilia;

32 centrioles were counted on a single section. The cilia are approximately as long as the cell is high. The basal bodies are stricly aligned, but their structure has not been studied in detail. A long rootlet departs at an angle of about 55° that descends almost down to the basal lamina. The striation from one center of a dark band to the next has an interval of 50-75 nm. The cells rest on a basal lamina of variable thickness. Towards the dorsal and ventral tips of the slit the basal lamina becomes thicker and forms, together with connective tissue fibers, a kind of supporting structure. Beyond the tips both layers, one from each side of the slit, unite to form short peduncles that attach on the outer body wall. In cross section this structure looks like the eye of a needle (Figs. 1E lower insert, 31). Sometimes there is direct contact between a ciliated cell and nervous tissue.

Sensory receptors occur proximal to the ciliary organ. They are probably arranged in two rows, separated by epithelial cells, but this remains to be confirmed. In many aspects these receptor cells resemble those of the anterior mantle edge of the Dentaliida. The nerve process is inflated in the epithelial region and the cytoplasm appears transparent. Five to eight cilia extend their short rootlets to apically located mitochondria. Many filaments and/or tubules can be found in this area. Towards the mesenchyme the cell tapers to a small process, but a direct connection to a perikaryon of a neuron could not be observed.

Distal to the ciliary organ the narrow slit widens to the base of the pavillon proper. The mantle appendage is, as in dentaliids, crescent-shaped in cross section (Fig. 33). Living animals, however, usually show the pavillon as a flat sheet of tissue, which can even be bent outwards. In the inner epithelium the flat cells are usually folded as well, but the large subepithelial gland cells are missing. Instead, secretory cells are present in the epithelium. Their cytoplasm contains various dark granules and vesicles. The apically located nuclei appear very condensed. Such dark and condensed nuclei are found frequently near the epithelium but completely detached from it. This indicates a holocrine secretion modus. Like in the dentaliid genera the pavillon crests are differentiated with regard to their epithelium. A continuous row of three to six ciliated cells run along the ledges (Figs. 33, 34). Cell shape ranges from cuboid to high-prismatic, with a height of 7-12 μm. The cilia of these cells are about 8 μm long and of normal structure. The basal apparatus consists of a centriole and, attached to it, a single striated rootlet at an angle of 20° to the ciliary axes. Between the cilia are microvilli of about 2 μm length. The cytoplasm stains faintly red with AZAN and HE. In TEM sections it appears relatively light and contains mitochondria, rough endoplasmic reticula (RER), golgi vesicles and other vesicles of various sizes and densities. The nucleus is spherical or elongated and situated close to the basal lamina. Frequently, nucleoli are present. At the tip of the pavillon sensory receptors can be recognized between the ciliated cells.
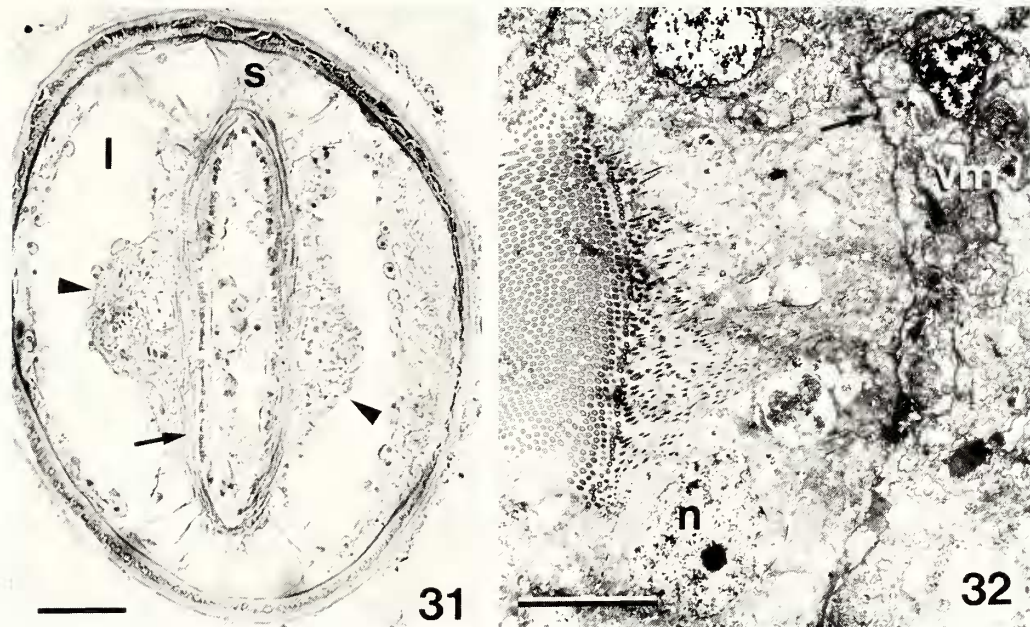
Fig. 31. *Entalina quinquangularis,* cross-section of the posterior mantle edge (formalin, H.E., 7 μm); l, lacunae; s, ventral socket of connective tissue; fibres of the valve muscle (arrows); crests of nerve- and glia cells (arrow-heads); scale bar = 50 μm. **Fig. 32.** *Entalina quinquangularis,* TEM micrograph of the valve ciliary organ of the posterior mantle edge, horizontal section (glutar-paraformaldehyde - OsO₄, Spurr); note the long ciliary rootlets descending between the densely packed mitochondria in the ciliated cell; n, nucleus of ciliated cell; vm, valve muscles; basal lamina (arrow); scale bar = 5 μm.
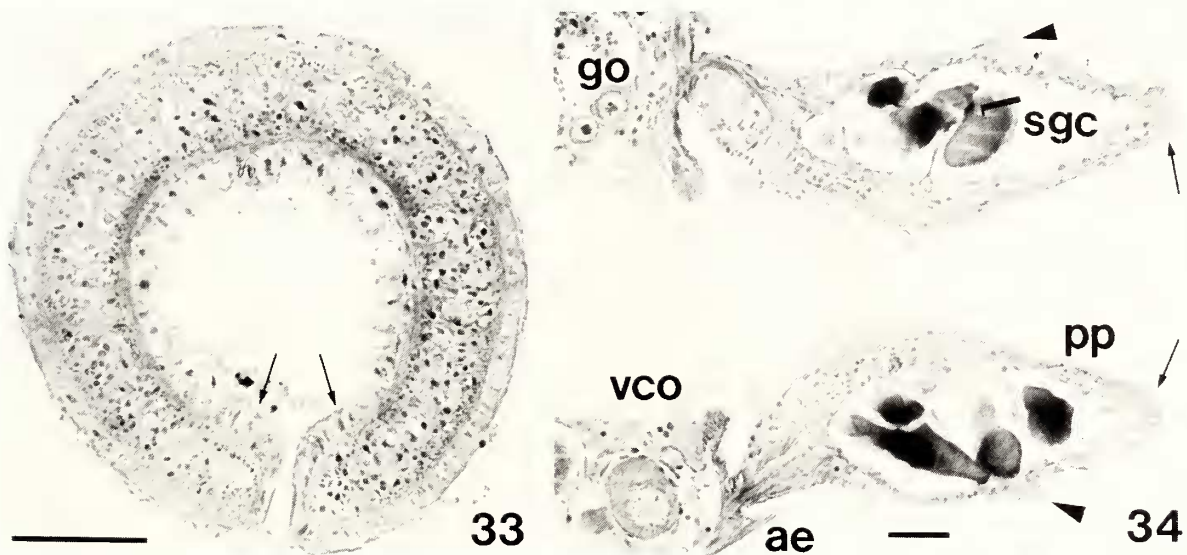

Fig. 33. *Entalina quinquangularis,* cross-section of the pavillon proper (Bouin, AZAN, 7 μm); ciliated ledge cells (arrows); scale bar = 50 μm. **Fig. 34.** *Cadulus subfusiformis,* longitudinal section of the posterior mantle edge (Bouin, AZAN, 5 μm); note the additional ring fold (arrow-heads) and the ciliated ledge cells (arrows); ae, attachment epithelium; go, gonad (ovary); pp, pavillon proper; sgc, subepithelial gland cells; vco, valve ciliary organ; scale bar = 25 μm.

They are inflated cell processes with the perikaryon below the epidermis. The cytoplasm looks empty. Elongated mitochondria are present close to the cell apex, and neurotubules extend vertically through the process. The cell surface shows only small microvilli, but it is provided with two to eight cilia with the common 9x2+2 pattern and a rootless centriole.

The outer pavillon epithelium is not as folded as the inner one; thus, its cells are of more constant cuboid shape and size (4-4.5 μm). The microvilli covering the surface are about 1.7 μm long. Numerous dark vesicles, mitochondria and RER are contained in the cytoplasm. The nucleus is close to the basal lamina. TEM reveals groups of two or three gland

cells in regular intervals. The dark cells closely resemble the secretory cells of the inner epithelium. All examined Gadilida possess an additional ring-fold distal and parallel to the supramarginal groove (Fig. 34).

Some of the gadilid species studied show subepithelial gland cells opening through the inner epithelium of the pavillon. The species are *Pulsellum lofotense* (M. Sars), *Wemersoniella turnerae* Scarabino, and all *Cadulus* species (Fig. 34) except *Cadulus sp.* (NMMH 803468) (Table 3).

DISCUSSION

FUNCTIONAL ASPECTS

The tubular nature of the peripedal pallial cavity can be considered a consequence of a rounding-off process of the mantle-shell-complex when adopting a burrowing habit (Pojeta and Runnegar, 1985; Runnegar and Pojeta, 1974; Runnegar, 1978; Salvini-Plawen, 1981, 1984). This process is recapitulated in scaphopod ontogeny (Lacaze-Duthiers, 1856-7; Kowalevsky, 1883). As in bivalves, the mantle cavity is not only the site of respiration, defecation and excretion, but also of food intake (Dinamani, 1964a, b). The organs of the mantle edges protect the animal against, and put it in touch with, its environment.

GLANDS

Experimental and detailed histochemical data on the functions of the various gland regions of the mantle epithelium

are lacking. Therefore, their functional interpretation must be based on structure and the observations on living animals alone.

The main function of the gland cells of the anterior mantle edge is probably mucus production. They closely resemble the mucus cells described in other marine invertebrates by Storch and Welsch (1972). This resemblance, the staining properties and the observations on living animals, indicate a mucoid nature of the secretions which have the same optical properties as water and can be discharged in copious amounts. Proteinaceous components may also be assumed, as the circum-nuclear cytoplasm is rich in rough endoplasmic reticulum. The basal portions of the cells are frequently associated with muscle fibers. Members of the order Dentaliida, where a prominent outer gland region is present, secrete enormous amounts of acid mucoid substances. The roles of the different types of gland cells are not clear. In *Antalis, Fustiaria* and *Rhabdus* the secretion is a sticky mass with the same optical properties as water. Gainey (1972) and Poon (1987) reported a feeding cavity in the sediment made by the foot after the animal is dug in. Gainey (1972) supposed that this cavity might be coated with mucus. At least for Dentaliida it is likely that this coating originates from the outer gland region of the anterior mantle edge. The weakly developed gland cells in the frontal epithelium of Gadilida are hardly capable of providing a mucus sheet to support a feeding cavity. In living *Entalina, Pulsellum* or *Cadulus* such massive mucus secretion has never

Table 3. Characters of the posterior mantle margin: (++) abundant; (+) present; (±) scarce, few; (−) absent; (?) no information.

	Aperture		Valve Mechanism								Pavillon		
	slit horizontal	slit vertical	ring sinus	dorsal flap	ventral bolster	subepith. gland cells	ciliated area	sphincter muscle	dorso-ventral constrictors	ciliary organ	ciliated ledges	subepith. gland cells	additional ring-fold
Antalis	+	−	+	+	+	+	+	+	−	−	−	±	−
Dentalium	+	−	+	+	+	+	+	+	−	−	−	+	−
Fissidentalium	+	−	+	+	+	+	+	+	−	−	−	±	−
Graptacme	+	−	+	+	+	+	+	+	−	−	−	±	−
Fustiaria	+	−	−	+	−	−	+	+	−	−	−	++	−
Laevidentalium	?	?	?	?	?	?	?	?	?	?	?	?	?
Rhabdus	+	−	+	+	+	+	+	+	−	−	−	±	−
Entalina	−	+	−	−	−	+	−	−	+	+	+	−	+
Bathoxiphus	−	+	−	−	−	±	−	−	+	+	+	−	+
Heteroschismoides	−	+	−	−	−	?	−	−	+	+	+	?	?
Pulsellum	−	+	−	−	−	+	−	−	+	+	+	±	+
Annulipulsellum	−	+	−	−	−	+	−	−	+	+	+	−	+
Striopulsellum	−	+	−	−	−	+	−	−	+	+	+	−	+
Siphonodentalium	−	+	−	−	−	+	−	−	+	+	+	−	+
Wemersoniella	−	+	−	−	−	±	−	−	+	+	+	++	+
Gadila	−	+	−	−	−	+	−	−	+	+	+	−	+
Cadulus	−	+	−	−	−	+	−	−	+	+	+	+	+

been observed.

Another function of the mucoid secretions of the anterior mantle edge appears to be the cleansing of the pallial opening from particles and the protection against disturbance by other organisms. The latter view is supported by the observation that moribund animals are invaded by protozoans, e.g. ciliates.

The inner gland region, common to all scaphopods, acts as a lubricator for the foot. This is not only important for locomotion and sealing of the pallial aperture but also for feeding. Gainey (1972) reported that the dentaliids *Antalis pseudohexagonum* Ihering (Henderson) and *Graptacme eboreum* (Conrad) collect food particles in the mantle cavity by ciliary action of the dorsomedian foot-furrow before they are ingested. The fact that gadilid scaphopods have epithelial as well as subepithelial gland cells in this region could point towards a compensation for the less elaborated outer gland region, compared to dentaliids.

The glandular region anterior to the preanal ciliary ridges has been regarded homologous with the hypobranchial gland of other molluscs by Distaso (1906). The absence of gills as a location reference and the lacking detailed structural analysis lend little weight to this hypothesis. However, it is likely that the mucoid secretion produced there lubricates fecal material and/or binds other particles in the mantle cavity before they are removed through one of its openings. It seems justified to assume that the subepithelial gland cells, which are most similar to those of the anterior mantle edge, secrete mucus for cleansing and repellant purposes.

Numerous authors (Simroth, 1894; Yonge, 1937; Stasek and McWiliams, 1973; Shimek, pers. comm.) ascribe the ability of shell reabsorption and truncation, as well as the formation of secondary apical shell features (pipes, plugs, lobes or slits) to the gland cells of the pavillon. Although this study provides no evidence for either of these functions, there are clear indications for the temporary nature of most apical shell features; they should therefore not be used as reliable systematic characters.

SENSORY RECEPTORS

The assumption that the receptor cells in the epithelia of the mantle margins have sensory qualities is based on their similarity with sensory cells of other invertebrates. The common charactes are: an electron lucent cytoplasm of almost empty appearance, the presence of one or more cilia with or without sunken-in bases, neurotubules in parallel orientation to the cell axis, a long, subepithelial, dendritic process, the absence of nuclei in the epithelial region, and, if present, the collar of eight to nine stereomicrovilli. Chemo- and mechanosensory functions are ascribed to these types of ciliary receptor cells.

Sensory cells similar to type 1 and 1a of the anterior mantle edge of *Antalis* and *Fustiaria* have been described for

the siphons of the bivalves *Donax serra* Röding, *D. sordidus* Hanley and *Solen capensis* Fischer by Hodgson and Fielden (1984, 1986), as well as for *Schizochilus caecus* L'Hardy (Neorhabdocoela, Kalyptorhyncha) by Ehlers and Ehlers (1977). The collar-receptor of type 2 is of a very common type found in numerous metazoan taxa (Haszprunar, 1985; Salvini-Plawen, 1988).

Structures bearing certain similarity with the papillae of the frontal epithelium of *Entalina* and *Pulsellum* are reported for the tentacles of the bivalve *Placopecten magellanicus* (Gmelin) by Moir (1977), although in the latter the papillae exhibit collar-receptors with nuclei.

The ciliary organ of the dentaliid anterior mantle margin probably has no sensory qualities. The vividly beating cilia in living animals suggest the function of water current generation. The length of the cilia, compared to the pallial orifice diameter, is not sufficient to produce an effective flow of water in the mantle cavity unless the opening is maximally constricted. Alternatively, the ciliary organ could be responsible for water exchange over the frontal epithelium and thus facilitate chemoreception.

The dorsolateral slits in the anterior mantle edge of *Rhabdus rectius* probably represent true sensory organs. This remains to be confirmed by TEM investigations.

Contrary to the opinion of Distaso (1906), no osphradial sense organs are developed in the epithelium beneath the visceral ganglia. Distaso may have misinterpreted the pre-anal ciliary ridges and the medullary nature of the visceral connectives in this area.

No detailed descriptions of the sensory structures of the posterior mantle edge of Scaphopoda have been published. Reynolds (1988) gave a short report on the occurrence of multiciliated receptors in *Rhabdus rectius* (Carpenter), but no comparison with the corresponding structures in *Entalina quinquangularis* (Forbes) can be made at present.

APICAL VALVE-ORGAN

ANTALIS - TYPE

The arrangement of muscles and connective tissue in *Antalis* spp. indicates that the width of the opening is regulated by the dorsal section of the annular fold, while the ventral part acts as an abutment for the musculature. The dorsal section closes the aperture when it is pressed against the ventral part and against the semi-annual projection on the dorsal side. The action of the sphincter alone is probably not responsible for this; blood pressure in the sinus certainly helps to close the valve. The muscle fibers transversing the dorsal section of the sinus open the valve.

FUSTIARIA - TYPE

Due to the lack of a ventral bolster and a sphincter muscle, rising blood pressure is likely to be the only force

lowering the large dorsal flap. Like in the *Antalis*-type, decreasing pressure and contraction of the dorsoventral muscle fibers lift the flap to open the pallial orifice.

ENTALINA - TYPE

The closure of the mantle opening to a vertical slit is probably effected by the dorsoventral muscle bundles on either side of the orifice. Dorsally and ventrally attached to connective tissue sockets, they constrict the orifice by approximating its lateral walls. Increasing haemolymph pressure in the surrounding lacunae is probably synergetic. The antagonistic force is provided by the radially arranged muscle fibers between inner and outer mantle epithelia.

WATER CIRCULATION

Respiration, defecation and excretion depend on the currents of water through the mantle cavity. Its surface has to meet the respiratory demands of the organism, as ctenidia are not present. Yonge (1937) gave a detailed account of the currents in the pallial cavity of *Antalis entalis*. He described the apical orifice as the inhalent and exhalent opening, observing a steady inflow of water due to ciliary action, followed by a violent expulsion, which is caused by a sudden retraction of the foot. In general, these results can be confirmed, although some complementary remarks can be made.

The inhalent water currents are produced by the ciliary organ of the pavillon and the preanal ridges in Gadilida, while in Dentaliida the ciliary beat of the more numerous preanal ridges alone generate the flow. The metachronal waves of the cilia can be easily observed through the transparent shells of *Rhabdus*, *Pulsellum* and *Cadulus*. Contrary to Yonge's reports, the apically entering water leaves the mantle cavity through the anterior aperture. This was observed in *Entalina quinquangularis*, *Pulsellum lofotense*, *Cadulus subfusiformis*, and *Antalis occidentalis* (Stimpson). Suspended carmine particles and sperm could be followed through the mantle cavity.

During the flow of water through the pallial cavity, the foot can only play a minor role in producing the inhalent current. The piston-like actions of the foot causing the rapid apical exhalent current occur about twice a minute in relaxed specimens of *Antalis* and *Fustiaria* (Dentaliida). The gadilid species showed intervals of one minute or more between expulsions. During the expulsions and during burrowing action the ciliary beat ceases; it is resumed a few seconds later.

SYSTEMATICS IMPLICATIONS

Systematics below the order level are solely based on shell and radula characters. Chistikov (1975a, b) attempted to include some criteria of the soft body.

The results of the present study provide detailed information on the differences of the mantle edges between the scaphopod orders, but also allow some statements at the family level.

DENTALIIDA

The anterior pallial edge of the order Dentaliida is characterized by a conspicuous outer gland region, a strong ring of cartilage-like material, as well as massive fibrous connective tissue and muscles in the central fold, a circular ciliary organ, and an inner gland region with epithelial gland cells only. The average number of preanal ciliary ridges ranges from 12 to 15.

In most representatives of this order the valve organ of the posterior mantle edge shows a dorsal and a ventral elevation of the pallial wall in combination with an annular blood sinus. Closing of the horizontal slit is achieved by lowering the dorsal flap by means of muscular action and haemolymph pressure. No ciliary tracts are developed on the ledges of the pavillon proper.

These features are shared by most of the examined dentaliid genera, such as *Antalis*, *Dentalium*, *Fissidentalium* and Laevidentalium. Thus, analysis of this organ system would suggest the grouping of these genera in the family Dentaliidae. With the exception of *Laevidentalium*, this grouping agrees with that of Scarabino (1979).

The genus *Fustiaria* differs from the general scheme in three aspects: 1) The radially arranged fibers of connective tissue in the central fold of the anterior mantle edge are not developed; 2) The relative abundances of the two types of sensory cells are completely different, and an additional kind of ciliary receptor is present (type 1a); 3) The most complex difference to the *Antalis*-type organization concerns the posterior mantle edge.

These differences, together with the particularities of the shell, the radula (Scarabino, 1979), and the foot (Steiner, in press), justify the separation of *Fustiaria* from the Dentaliidae. Thus, a new monogeneric family, the Fustiariidae, is proposed.

FUSTIARIIDAE (FAM. *NOV.*)

DIAGNOSIS — Dentaliid Scaphopoda with smooth, polished, thin-walled and transparent shells of slender shape, moderature curvature and circular cross-section. The ventral side of the apex can have a straight, deep slit or a small notch. The rachis tooth of the radula differs from that of Dentaliidae by showing a flat superior edge. The ciliary organ of the anterior pallial margin consists of five to six rows of cells. The posterior mantle orifice can be closed by a dorsal flap. No ventral bolster, sphincter muscle or glands are elaborated there. Instead, subepithelial gland cells are abundant in the pavillon. The pedal sinus is divided by a horizontal septum.

TYPE GENUS — *Fustiaria* Stoliczka, 1868. The genus *Rhabdus*, represented by *R. rectius* in this study, has a

remarkable specialization at the anterior mantle margin. A pair of dorso-lateral invaginations at the periphery of the outer gland region are developed, and the ciliary organ is wanting. The slit-like invaginations can either be considered denovo differentiations of the outer gland replacing the lost ciliary organ, or a differentiation of the ciliary organ itself.

The family Rhabdidae was erected by Chistikov (1975a), who emphasized the peculiar morphology of radula and shell. The special elaboration of the anterior pallial edge supports the separation from the other dentaliid Scaphopoda.

GADILIDA

The anterior mantle edge of Gadilida differs from the corresponding organ of Dentaliida by the lack of an outer gland region and the ciliary organ. The frontal epithelium is elaborated into papillae which carry ciliary sensory cells. The amount of cartilage-like and fibrous connective tissue in the central fold varies species-specifically. The inner gland region exhibits subepithelial as well as epithelial gland cells. The average number of ciliary rings in the preanal area (4 to 8) is lower than in the Dentaliida.

The posterior pallial edge is characterized by a powerful ciliary organ of large ciliated cells arranged in a single row encircling the mantle opening. Dorsoventral muscle fibers close the aperture by approximating the lateral walls of the pallial opening, thus producing a vertical slit. No distinct annular blood sinus is developed. The ledges of the pavillon proper are lined by ciliary tracts.

At present the only anatomical character suitable for a classification within the Gadilida is the different abundances of papillae in the frontal epithelium of the anterior mantle edge. These structures were found in great numbers in the examined species of *Entalina, Bathoxiphus, Heteroschismoides, Pulsellum, Annulipulsellum, Striopulsellum* and *Wemersoniella.* Comparatively few papillae are developed in *Siphonodentalium, Cadulus* and *Gadila.*

For the common ancestor of Dentaliida and Gadilida the following differentiations of the mantle can be assumed: 1) a rigid ring formed by connective tissue and muscles to support the anterior pallial opening, 2) an area of epithelial gland cells inside the mantle cavity (= inner gland region), 3) several ciliated, annular ridges in the preanal region, perhaps preceded by a ventral glandular field (=hypobranchial gland of Distaso), 4) the posterior mantle opening with an elaborated dorsal extension, the pavillon proper.

According to this list, the outer gland region and the presence of a ciliated organ at the anterior pallial aperture are differentiations within the Dentaliida, while the subepithelial gland cells of the inner gland region and the ciliation of the pavillon proper have developed in Gadilida only.

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